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Plasma Acetyl-Carnitine Concentrations during and after a Muscular Exercise Test in Patients with Liver Disease

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Summary: In many human tissues, fuel is stored for immediate use, as well as for energy exchange between different parts of the body. Fat and glycogen represent, together with proteins, the principal energy storage materials. During energy requirement, e. g. muscular exercise, glycogen as a local reserve, is used first to supply energy needs. Acetyl-carnitine, as an active molecular group, represents an intermediate substrate, usable directly in the working tissue. The present study investigates whether plasma acetyl-carnitine could be a useful biochemical measure for information on fuel exchange in the body, and whether it is a rapidly available energy source exchangeable among tissues with different metabolic functions, such as muscle and liver. The present study investigated control and hepatopathic subjects after maximal and submaximal muscular exercise. Hepatopathic patients may be a useful model, as liver carnitine metabolism is likely to be impaired. Plasma acetyl-carnitine before, during and after maximal exercise in hepatopathic subjects did not differ, while in normal subjects it increased. After submaximal exercise, acetyl-carnitine increased in patients, as well in controls. In the patients ($n = 9$) with liver metabolism disorders we observed that during maximal exercise plasma acetyl-carnitine varied from 3.26 ± 2.18 μmol/l (time 0 min) to 4.30 ± 2.02 μmol/l (time 20 min) and from 1.99 ± 1.36 μmol/l to 4.83 ± 2.60 μmol/l ($p < 0.05$) in the controls ($n = 7$). During submaximal exercise, plasma acetyl-carnitine varied from 1.89 ± 1.7 μmol/l (time 0 min) to 2.92 ± 2.22 μmol/l (time 30 min) ($p < 0.05$) and to 4.32 ± 2.22 μmol/l (recovery, time 90 min) in controls, while in patients there was a significant variation from 2.98 ± 1.52 μmol/l (time 0 min) to 5.98 ± 1.43 μmol/l (recovery, time 90 min). Although plasma acetyl-carnitine concentrations were related to tissue metabolism variations, the relationships of substrate fuel exchange to tissue carnitine metabolism between different parts of the body are still unclear.

Introduction

Glucose, lipids and amino acids are the principal sources of energy during exercise. All metabolic processes depend on an intermediate acetyl group, principally bound to coenzyme A to form acetyl-coenzyme A. Many researchers have investigated the regulatory factors influencing one of the substrates (carbohydrates, lipids or amino acids) relative to the metabolic state.

The carnitine "shuttle" mechanism, by which fatty acids are transported across the mitochondria membrane, can produce acyl-carnitine esters and it is essential in lipid catabolism (1). Of the different esters acetyl-carnitine without doubt holds an important position as a molecule able to subtract acetyl (carnitine as acceptor for acetyl units) from acetyl-coenzyme A, while maintaining a viable pool of coenzyme A, as well as storing "active acetate", readily available for trans-acetylation back to acetyl-coenzyme A (2–4).

Investigation of carnitine and acyl-carnitine metabolism in different types of tissue has produced very interesting results. Considerable difficulties, however, are encountered in investigating the role of this sub-

strate in human tissue and in particular in the whole-body model, due to the relationships between different tissues and organs. Numerous scientists have therefore studied the variations occurring in different physiological and pathological states (5–8).

Other authors (9) and our laboratory (10) have demonstrated variations in plasma free carnitine and acetyl-carnitine during physical exercise. Acetyl storage and utilization, where appropriate, also seem to be linked to carnitine. *Lennon* et al. (9) observed that carnitine palmitoyl transferase activity varied in muscle tissue during training. These observations seem in agreement with the hypothesis that carnitine is involved at different levels in various tissue functions.

Plasma esterified and free carnitine concentrations may change in relation to the different tissue substrate exchange (energy-transfer), and particularly between liver and muscle. *Lennon & Mance* (11) studied interorgan cooperativity and carnitine metabolism, particularly carnitine acetyl- and palmitoyl-transferase activity in a variety of tissues of female rats, such as heart, liver, kidney, different skeletal muscle types and plasma. They concluded, after determining the effects of chronic endurance training, that an interorgan shift in carnitine distribution occurs. These authors proposed that the carnitine status is important in several human diseases. This type of research indicated that carnitine (with acetyl-carnitine) metabolism changes with the physical state of the organism. Physical exercise can therefore be used experimentally to influence carnitine metabolism. In fact acetyl-carnitine plasma concentrations increase in both maximal and sub-maximal performance (5, 8, 10).

It is known that the liver is a key apical organ in fat biosynthesis, mobilization and processing, and that carnitine is an important intermediate in these metabolic processes. Evidence is lacking, however, for cooperativity between the liver and other tissues (such as adipose and muscle tissues) with respect to the utilization of carnitine and the acetyl group. We therefore investigated plasma acetyl-carnitine variations in a pathological state involving altered hepatic function. We studied the effects of exercise on free carnitine and acetyl-carnitine plasma concentrations in liver diseases such as hepatic cirrhosis, in which a varied acetyl-carnitine metabolism is supposed (12–15), and during physical exercise, which results in plasma carnitine metabolism variations. The chronic persistent hepatopathic patient, without important handicaps which impede muscular exercise, and able to sustain a physical performance at exhaustion, could be a suitable model. Thus this type of subject could provide a model for studying carnitine and energy substrate exchange between liver and other tissues. The literature contains only limited data on the capacity for energy fuel substrate exchange during and after moderate physical exercise performance in these subjects (16). We therefore investigated controls ("normal", without illness in relation to metabolism) and chronic persistent hepatitis subjects before, during exercise and recovery, to verify whether energy requirements modify free carnitine and acetyl-carnitine plasma concentrations, and whether these variations could indicate an interorgan exchange in these two types of subject.

Materials and Methods

Subjects

Chronic hepatitis patients

Nine male patients with chronic persistent hepatitis, mean age 30 years (range 18–43 years) entered the study. The diagnosis of chronic persistent hepatitis was confirmed histologically in each case according to *De Groote*'s classification (17); impairment of liver function was also studied by measuring the increased concentrations of bilirubin (total and conjugated), alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transferase (tab. 1).

Control patients

Seven male healthy subjects, mean age 27 years (range 26–30 years) were selected as controls. They were recruited among the laboratory staff, were sedentary, with no regular practice of sport, and following a well-balanced diet.

Control subjects and chronic persistent hepatitis patients were voluntary, and informed of the nature and purpose of the study.

Exercise protocols

All subjects were kept on a low carnitine balanced diet (ca. 10 MJ/d ≙ 2400 kcal/d; 60% carbohydrate, 25% fat, 15% protein) 48 h before the test. In the morning, after an overnight fast, they performed a maximal (to exhaustion) exercise on a cycloergometer (model 300 Quiton Instrument) with 25 Watt energy load every 2 min. The exercise was continued for 20 min unless the subject was unable to continue because of symptoms of fatigue. A week later, under the same conditions, they performed a second submaximal test (50% of their previously determined maximal oxygen consumption [V_{O_2}max]) for 60 min.

Collection of blood samples

Blood samples (maximal excercise) were drawn at rest (at 0 min), at exhaustion (at 20 min, end of exercise) and at 30 min after the end of exercise (at 50 min, recovery). Blood samples (submaximal exercise) were drawn at rest (at 0 min), at 30 min, and at 60 min (the end of exercise) and at 30 min post-exercise (at 90 min, recovery).

Suitable sodium heparinized blood, was centrifuged and plasma was stored at −20 °C until analysis for free fatty acids, carnitine, acetyl-carnitine, lactate, and insulin. For glucagon analysis 2.5 ml of blood were collected in 0.25 ml of aprotinin (Trasylol®)/EDTA solution (500 · 10^3 KIU/120 g per l). One millilitre of blood was immediately deproteinized in 1.0 ml perchloric acid (1 mol/l) for measurement of the β-hydroxybutyrate concentration.

Tab. 1. Routine serum chemistries, determined in reference subjects, ranges are
L-aspartate : 2-oxoglutarate aminotransferase, 0–45 U/l,
L-alanine : 2-oxoglutarate aminotransferase, 0–50 U/l,
γ-glutamyl-peptide : aminoacid γ-glutamyltransferase, 3–65 U/l,
orthophosphoric monoester phosphohydrolase, 30–115 U/l,
bilirubin (total) 2.0–17 μmol/l, (direct) 0.0–3.4 μmol/l.
Data expressed as mean values (± SEM); n, number of subjects

	Aspartate amino-transferase U/l (*)	Alanine amino-transferase U/l (*)	Total bilirubin μmol/l (*)	Direct bilirubin μmol/l (*)	γ-Glutamyl-transferase U/l (*)	Alkaline phosphatase U/l (*)
Controls (n = 5)	22 (2)	28 (4)	10.3 (1.1)	0.54 (0.35)	23 (6)	52 (6)
Hepatopathics (n = 5)	76 (20)	152 (55)	17.5 (3.5)	3.08 (1.0)	47 (7)	78 (10)

(*) $p < 0.05$ control vs hepathopatic subjects; statistical comparisons were made using *Student*'s t-test.

Assay methods

Plasma free fatty acids, β-hydroxybutyrate, glucose, lactate were assayed by conventional enzymatic methods (18–20). Plasma insulin and glucagon concentrations were assayed by conventional radioimmunoassay (RIA) with commercial kits purchased from Sorin Biomedica and BYK, respectively. Serum bilirubin and enzymes were assayed according to routine laboratory methods and according to the recommendation of IFCC Expert Panel for Enzymes at 37 °C. Plasma free carnitine and acetyl-carnitine were analysed by an enzymatic assay as previously described (21). Plasma sample (1.0 ml) was placed on a minicolumn (55 × 15 mm Sephadex-G25), and the column eluted with assay buffer. Two millilitres of sample (6th and 7th 1.0 ml elution fractions) were collected and divided into two aliquots (1.0 ml each) for direct free carnitine assay, or lyophilized for acetyl-carnitine assay.

Free carnitine assay

Acetyl-CoA (15 mmol/l) 0.05 ml, 5,5′-dithiobis(2-nitrobenzoic)acid (10 mmol/l) 0.01 ml, and ethylenedinitrilo tetra-acetic acid (EDTA, 50 mmol/l) 0.025 ml were added to each eluted sample (0.900 ml). After 10 min at the constant temperature of 25 °C, carnitine acetyl-transferase (EC 2.3.1.7 – 80 kU/l) (0.010 ml) was added to start the reaction and absorbance was measured at 412 nm. Reaction equilibrium was reached after 7 min and the absorbance (after 30 min) was referred to a standard curve (linear throughout the concentration assayed range 10–50 μmol/l). For the plasma free carnitine assay, the within-batch imprecision calculated (using replicated analysis) from duplicate estimation of patient samples, was 4%; the between-batch imprecision, similarly calculated, was 9%. The mean concentration in healthy subjects was 33 ± 2 μmol/l (mean ± SEM, n = 19). The assay has a minimum detection limit of 5 μmol/l estimated from 3 standard deviations of a sample without carnitine.

Acetyl-carnitine assay

Malic acid (100 mmol/l) 0.010 ml, EDTA (25 mmol/l) 0.0125 ml, nicotinamide adenine dinucleotide (NAD, free acid, grade II, 98%, 5 mmol/l) 0.025 ml, coenzyme A (CoA-SH free acid, grade I, lyophilisate, 5 mmol/l) 0.005 ml, malate dehydrogenase (EC 1.1.1.37 from pig heart mitochondrial suspension, 1 g/l, 1.2 kU/l) 0.005 ml, citrate synthase (EC 4.1.3.7 crystallized 1 g/l, 110 kU/l) 0.005 ml, were mixed to form a medium and 0.070 ml of this medium was added directly in a cuvette to sample (0.500 ml) previously prepared by regeneration of lyophilized powder. Fluorescence intensity was then measured and 0.010 ml carnitine acetyl-transferase (80 kU/l, 1 g/l) was added to start the reaction. It was then possible to measure fluorimetrically (excitation 337 nm; emission 457 nm) the newly formed NADH in the solution. Equilibrium was reached after 7 min and the fluorescence intensity variations were referred to a standard curve (linear throughout 2.5–20.0 μmol/l). For the acetyl-carnitine assay, the within-batch imprecision calculated from duplicate estimation of patient sample, was 11%; the between-batch imprecision, similarly calculated, was 14%. The mean concentration in healthy subjects was 2.5 ± 0.2 μmol/l (mean ± SEM, n = 21). The assay has a minimum detection limit of 1.4 μmol/l estimated from 3 standard deviations of a sample without acetyl-carnitine.

The reagents used were of the highest purity commercially available (analytical grade).

Statistical analysis

All values are reported as means ± standard deviation (SD) or standard error of the mean (SEM). Statistical significance (defined as $p < 0.05$) of differences between different suitable groups was assessed by analysis of variance and by t test.

Results

In patients with chronic hepatitis, concentrations of blood glucose before, during, and after maximal muscular exercise did not differ significantly from those of normal subjects (tab. 2 and 3), although normal individuals showed higher concentrations than individuals with liver disease after maximal exercise.

In controls, concentrations of plasma lactate after maximal exercise were significantly higher than in the hepatitis group (fig. 1). After prolonged submaximal exercise, concentrations of plasma lactate did not differ significantly between the groups.

Before, during and after maximal exercise, plasma free fatty acids did not differ between the groups (fig. 2). After submaximal exercise, concentrations increased significantly in both groups (fig. 2), although plasma free fatty acid concentrations of individuals

Tab. 2. Concentrations of plasma lactate (mmol/l), free carnitine (μmol/l), acetyl-carnitine (μmol/l), free fatty acids (μmol/l), glucagon (μg/l), insulin (mU/l) and blood β-hydroxybutyrate (mmol/l), glucose (mmol/l) measured in control subjects before, during, and after maximal and submaximal muscular exercise. Data expressed as mean value ± SD; n, number of subjects.

		Maximal exercise group Times (min)			Submaximal exercise group Times (min)			
		0	20	50	0	30	60	90
Plasma lactate	mean	1.75	15.39[a]	7.90[a,b]	1.75	2.69	1.45	1.25
	± SD	0.36	2.02	1.26	0.58	1.34	0.69	0.62
	n	5	5	5	5	5	5	5
Plasma free carnitine	mean	24.00	22.60	18.20	28.00	25.80	25.00	28.00
	± SD	5.29	3.98	6.05	4.69	2.64	5.18	3.85
	n	5	5	5	5	5	5	5
Plasma acetyl-carnitine	mean	1.99	4.83[a]	4.93	1.89	2.92[a]	2.95	4.32[a,b]
	± SD	1.36	2.60	3.39	1.70	2.09	2.22	2.22
	n	7	7	7	6	6	6	6
Plasma free fatty acids	mean	321	304	365	398	304[a]	499	614[a]
	± SD	144	137	95	138	109	143	288
	n	7	7	7	7	7	7	7
Plasma glucagon	mean	–	–	–	110	108	111	120
	± SD	–	–	–	53	69	69	78
	n	–	–	–	7	7	7	7
Plasma insulin	mean	–	–	–	10	7[a]	6[a]	8
	± SD	–	–	–	4	2	3	4
	n	–	–	–	7	7	7	6
Blood β-hydroxybutyrate[d]	mean	0.07	0.09	0.12	0.06	0.07	0.06	0.18
	± SD	0.05	0.07	0.07	0.02	0.05	0.04	0.15
	n	7	7	7	7	7	7	7
Blood glucose	mean	4.46	5.09	5.49	4.44	4.05	3.97	4.37
	± SD	0.87	1.18	1.54	0.50	0.45	0.50	0.53
	n	7	7	7	7	7	7	7

[a] $p < 0.05$ vs basal
[b] $p < 0.05$ vs time 20 min
[c] $p < 0.05$ vs time 60 min
[d] $p < 0.05$ anova test (submaximal exercise test)

with liver disease were slightly, but not significantly, higher than those in control subjects.

The blood β-hydroxybutyrate concentrations, as shown in figure 3, decreased, but not significantly, during maximal exercise in hepatitis subjects, whereas during submaximal exercise the increase was evident in both groups only during recovery.

Before, during and after maximal and submaximal exercise, plasma free carnitine did not differ between the two groups (tab. 2 and 3) and did not vary significantly within the same group.

Plasma acetyl-carnitine before, during and after maximal exercise in hepatitis subjects did not differ, while in normal subjects it increased (fig. 4). After submaximal exercise, acetyl-carnitine increased in both the chronic hepatitis subjects and the controls.

Plasma insulin concentrations, shown in tables 2 and 3, were significantly different ($p = 0.05$) between controls and patients, while plasma concentrations were significantly decreased only for controls; glucagon concentrations also were higher in patients but not significantly (tab. 2 and 3).

Discussion

It is generally agreed that plasma free fatty acids are the primary fuel source for energy metabolism when glucose is not readily available, as in patients with diminished hepatic glycogen storage, i.e. in cirrhotic patients (22). The present data are in accordance with this view; plasma free fatty acid concentrations were in patients clearly higher at the end of submaximal exercise and during recovery (tab. 2 and 3). Moreover, increasing blood glucose concentrations during maximal exercise were observed in controls, but not in hepatopathic subjects; although this difference was not statistically significant, it does suggest defective

Tab. 3. Concentrations of plasma lactate (mmol/l), free carnitine (μmol/l), acetyl-carnitine (μmol/l), free fatty acids (μmol/l), glucagon (μg/l), insulin (mU/l), blood β-hydroxybutyrate (mmol/l), and blood glucose (mmol/l) measured in patients before, during, and after maximal and submaximal muscular exercise. Data expressed as mean values ± SD; n, number of subjects.

		Maximal exercise group Times (min)			Submaximal exercise group Times (min)			
		0	20	50	0	30	60	90
Plasma lactate	mean	1.63	8.89[a]	6.05[a,b]	1.56	3.06	1.87	1.46
	± SD	0.87	2.05	2.22	0.79	1.24	0.77	0.57
	n	9	9	9	9	9	9	9
Plasma free carnitine	mean	21.40	18.60	26.40	25.00	25.20	20.00	27.60
	± SD	7.47	5.54	6.05	7.01	6.79	4.52	7.14
	n	5	5	5	5	5	5	5
Plasma acetyl-carnitine	mean	3.26	4.09	4.30	2.60	2.79	3.44	5.98[a]
	± SD	2.18	2.23	2.02	1.30	1.89	1.31	1.43
	n	9	9	9	5	5	5	5
Plasma free fatty acids	mean	336	336	375	472	393	699[a]	698[a]
	± SD	103	86	182	133	147	295	181
	n	9	9	9	9	9	9	9
Plasma glucagon	mean	–	–	–	113	120	136	124
	± SD	–	–	–	32	39	37	33
	n	–	–	–	9	9	9	9
Plasma insulin	mean	12	14	14	14	9	7	7
	± SD	2	7	3	9	4	3	4
	n	4	4	4	9	9	9	9
Blood β-hydroxybutyrate[d]	mean	0.05	0.02	0.08	0.08	0.07	0.14	0.26
	± SD	0.06	0.02	0.05	0.05	0.04	0.11	0.20
	n	5	5	5	7	7	7	7
Blood glucose	mean	4.24	4.16	4.53	4.38	3.90	3.93	4.07
	± SD	0.72	0.64	0.88	0.42	0.53	0.54	0.75
	n	9	9	9	9	9	9	9

[a] $p < 0.05$ vs basal
[b] $p < 0.05$ vs time 20 min
[d] $p < 0.05$ anova test (submaximal exercise test)

liver gluconeogenesis as proposed by *Campillo* et al. (6).

Plasma free carnitine concentrations showed no variations, while it seems confirmed that plasma acetyl-carnitine was, even if not significantly, elevated in patients (controls: 1.94 ± 1.53 μmol/l (n = 13); patients: 3.02 ± 1.94 μmol/l (n = 14); all values for times 0 min of maximal and submaximal tests).

Plasma free carnitine concentrations in hepatopathic patients, studied by other authors, are different and widely distributed (12–15). Some of these results possibly are in disagreement with our results, because our patients did not have a severe hepatic dysfunction, and furthermore because the nutritional status was different. We measured plasma free carnitine and acetyl-carnitine in patients with chronic hepatitis without advanced liver disease or malnutrition, and who had followed a prescribed diet three days before the study. Discrepancy among different results might also be due to the use of different analysis methods and to the different nutritional states of subjects. In fact spectrophotometric methods were used in some laboratories, and radioenzymatic in others, to determine free carnitine and acetyl-carnitine plasma concentrations.

A significant increase of plasma free fatty acid concentrations at the end of the submaximal exercise and during the recovery confirmed that the energy supply consisted of lipids, both in controls and in patients.

Subjects with chronic liver disease undergoing maximal exercise showed impaired carnitine metabolism; their plasma acetyl-carnitine concentrations did not increase with exercise, as in controls ($p < 0.05$ basal vs 20 min), probably because of a decreased metabolism of carbohydrates in the liver. This also probably explains why, during maximal exercise, plasma lactate in patients increased less than in control subjects. Present observations seem in agreement with *DeLissio*

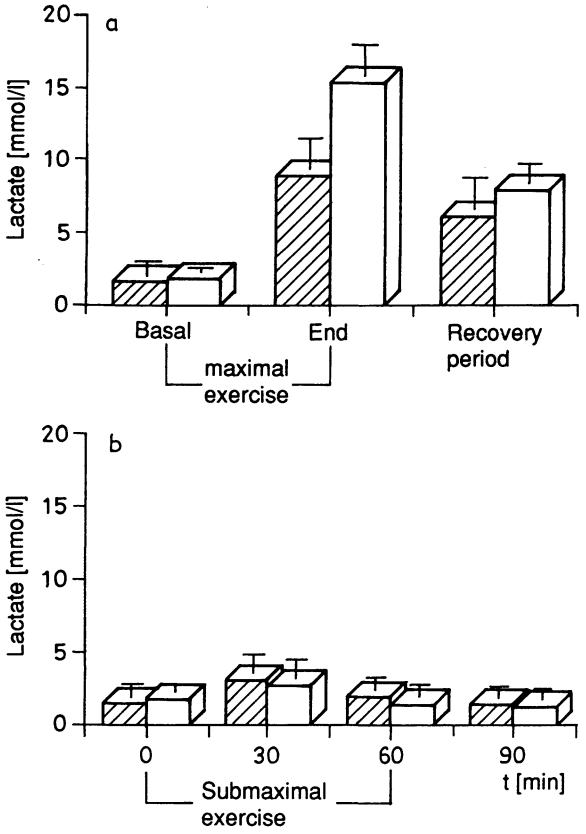

Fig. 1. Concentrations of plasma lactate before, during, and after maximal (a) and submaximal (b) muscular exercise. Data expressed as mean values ± SD.
▨ = Patients, □ = controls

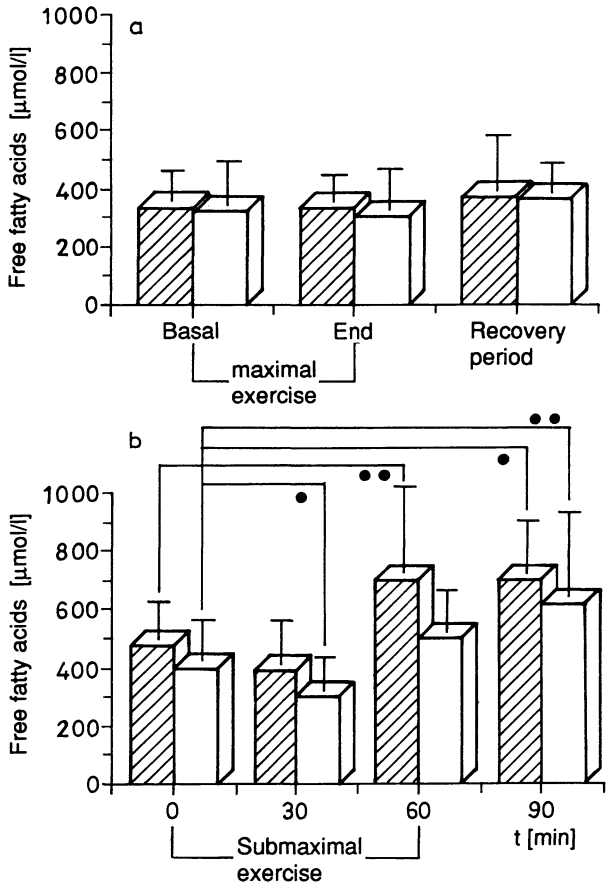

Fig. 2. Plasma free fatty acid concentrations before, during and after maximal (a) and submaximal (b) exercise. Data expressed as mean values ± SD;
● $p < 0.05$; ●● $p < 0.01$.
▨ = Patients, □ = controls

(16) who verified that, during exercise, cirrhotic subjects derived a significantly greater percentage of their total energy expediture from lipid oxidation and less from glucose oxidation, compared with the control subjects. The fact that our results do not agree with other previous observations (6) could be related to a different type of patient and a different experimental protocol. In fact our patients had only very slightly increased insulin plasma concentrations.

We measured the decreased plasma β-hydroxybutyrate concentrations in relation to their utilization in peripheral tissue; lower β-hydroxybutyrate concentrations during maximal exercise reflect increased oxidation of this ketone body, because it provides a good substrate for muscle metabolism during exercise, but it also indicates agreement with the fact that glycolysis is less active in these patients than in controls.

Our subjects were studied during muscular exercise, in which plasma free carnitine decreased and plasma acetyl-carnitine increased as a consequence mainly of activation of fat oxidation (2, 5, 18). On the other hand, *Lennon* et al. (11) demonstrated in rats an interorgan shift in carnitine distribution in response to chronic training, suggesting that the majority of acyl-carnitines formed can be transported, during muscular exercise, to the liver for glucose or ketone body formation.

Carlin et al. (5) proposed that the mechanism responsible for the increased plasma acetyl-carnitine in maximal muscular exercise could partly be explained by the different hormonal profiles (6). During submaximal exercise, our hepatopathic subjects showed no significant plasma insulin concentration variations, and the pattern of acetyl-carnitine was the same as in normal subjects.

Conclusion

The different acetyl-carnitine concentrations during maximal and submaximal exercise in the two groups and in the two types of exercise, could be explained on the basis of the hypothesis that the increase in

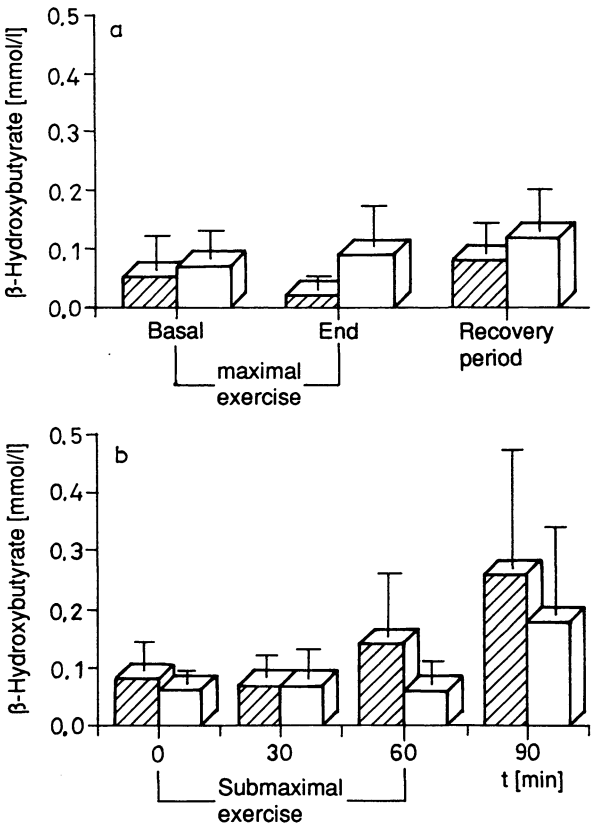

Fig. 3. Blood β-hydroxybutyrate concentrations before, during, and after maximal (a) and submaximal (b) muscular exercise. Data expressed as mean values ± SD.
▨ = Patients, □ = controls

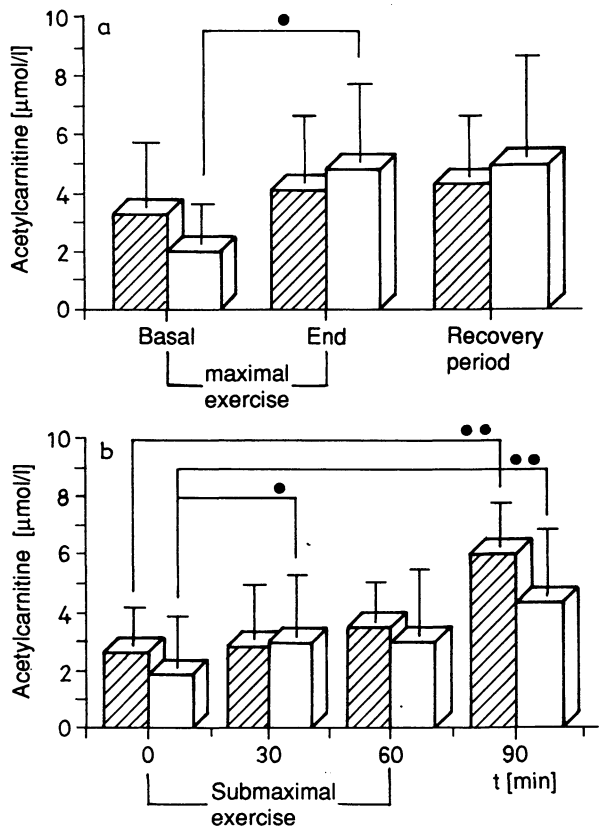

Fig. 4. Plasma acetyl-carnitine concentrations before, during, and after maximal (a) and submaximal (b) muscular exercise. Data expressed as mean values ± SD;
● $p < 0.05$; ●● $p < 0.01$.
▨ = Patients, □ = controls

plasma acetyl-carnitine is related to its exchange between muscle and liver. During both maximal and submaximal exercise, the patients showed a lower acetyl-carnitine concentrations variation than controls, who demonstrated a significant increase (0 vs 20 min $p < 0.05$), possibly due to a higher production. With submaximal exercise, hormonal control is more effective, and it is evident that free fatty acids are utilized by the livers of both controls and patients (23). These observations seem in agreement with the hypothesis that acetyl-carnitine is exchanged between muscle and liver via the blood circulation (24). For a complete knowledge of this inter-organ cooperation more investigation is needed, particularly about metabolite turnover, liver blood flow and muscle blood flow.

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